NTP
National Toxicology Program

Tox21

Overview of the Tox21 Collaboration

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National Institute of Environmental Health Sciences

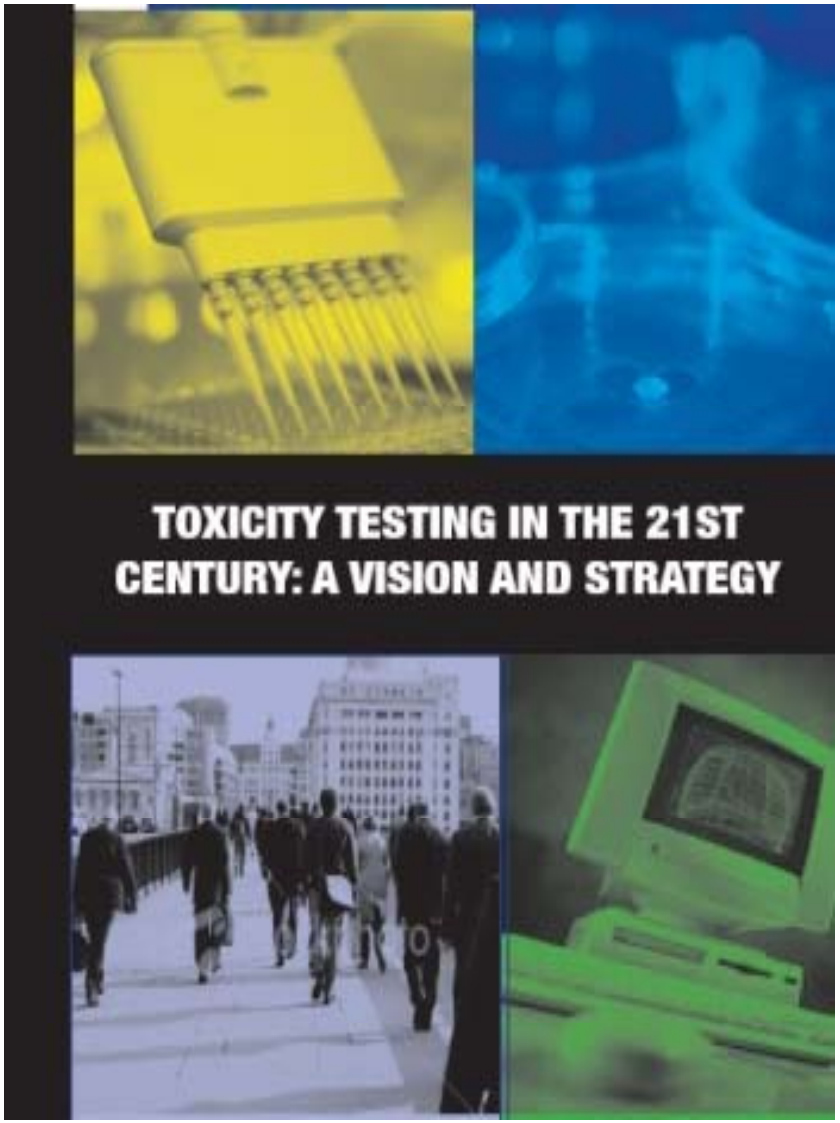
NTP Workshop: Role of Environmental Chemicals in the Development of Diabetes and Obesity

January 11-13, 2011

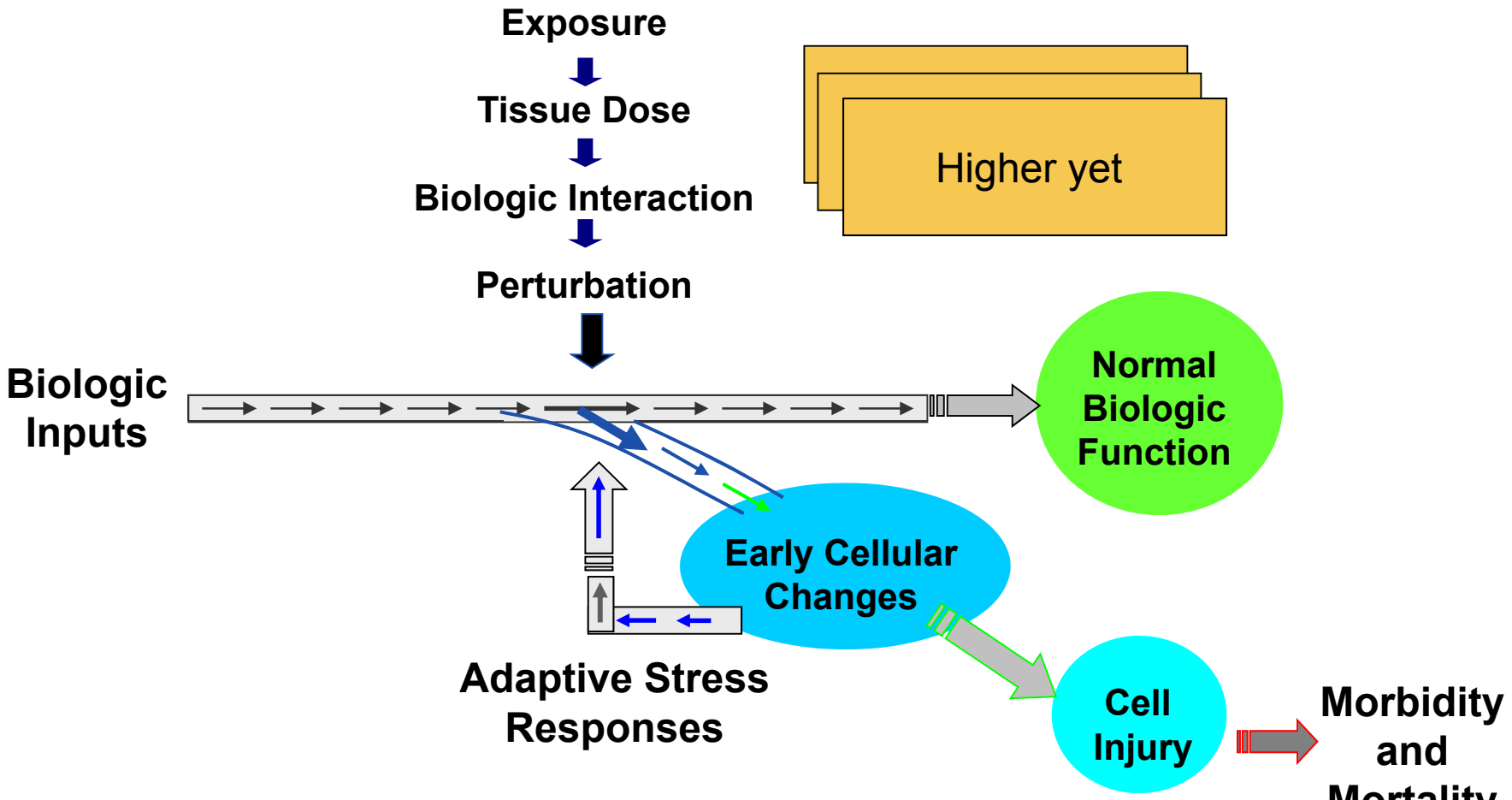
Raleigh, North Carolina

NTP Vision for the 21st Century (2004)

"To support the evolution of toxicology from a predominantly observational science at the level of disease-specific models to a predominantly predictive science focused upon a broad inclusion of target-specific, mechanism-based, biological observations."



This 2007 National Academy of Science report envisions a not-so-distant future in which virtually all routine toxicity testing would be conducted *in vitro* in human cells or cell lines by evaluating perturbations of cellular responses in a suite of toxicity pathway assays using high throughput robotic assisted methodologies.

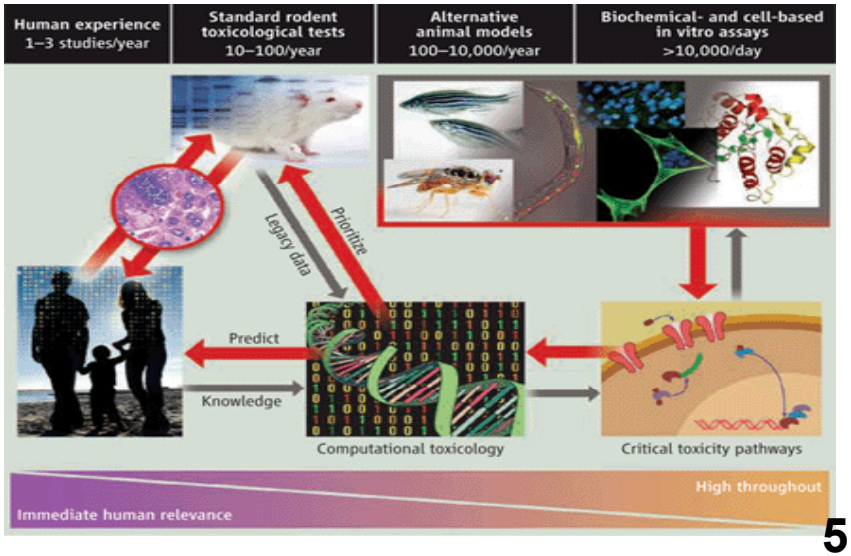


Activation of Toxicity Pathway

NAS Report, 2007

Memorandum of Understanding: "High-Throughput Screening, Toxicity Pathway Profiling and Biological Interpretation of Findings"

- Released February 14, 2008, as an agreement between:
  - National Institute of Environmental Health Sciences/National Toxicology Program
  - National Human Genome Research Institute/ NIH Chemical Genomics Center
  - U.S. Environmental Protection Agency/ Office of Research and Development



FS Collins, GM Gray, JR Bucher *Transforming Environmental Health Protection*, Science 319, 906, 2008 (Toxicology Policy Forum)

Formation of an Expanded U.S. Tox21 Community

- Revised MoU on "High-Throughput Screening, Toxicity Pathway Profiling and Biological Interpretation of Findings" released on **July 19, 2010** (http://ntp.niehs.nih.gov/go/28213) by:

  - **National Toxicology Program:** Linda S. Birnbaum, Ph.D., DABT, ATS Director, National Institute of Environmental Health Sciences, National Institutes of Health
  - **NIH Chemical Genomics Center:** Eric D. Green, M.D., Ph.D. Director National Human Genome Research Institute
  - **U.S. Environmental Protection Agency:** Paul T. Anastas, Ph.D. Assistant Administrator Office of Research and Development
  - **Food and Drug Administration:** Janet Woodcock, M.D., Director Center for Drug Evaluation and Research

Tox21 Partners

NTP: Raymond Tice, Jennifer Fostel, Cynthia Smith, Kristine Witt
C. elegans Screening Core: Jon Freedman, Windy Boyd, Paul Dunlap, Julie Rice

NIH Chemical Genomics Center

Chris Austin, Ruili Huang, David Gerhold, Noel Southall, Menghang Xia

National Center for Computational Toxicology: Bob Kavlock, David Dix, Keith Houck, Richard Judson, Ann Richard

U.S. Food and Drug Administration
Protecting and Promoting *Your* Health

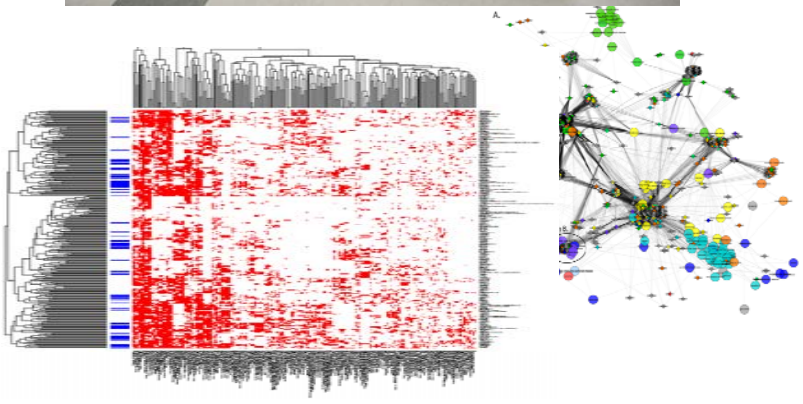
David Jacobson-Kram, Dan Benz, Kevin Gaido, Donna Mendrick, Weida Tong

Key Provisions of the Tox21 MOU

- The MOU builds on existing expertise and overcomes the resource limitations of a single agency.
- The partners agree to collaborate on the research, development, validation, and translation of new and innovative test methods that characterize key steps in toxicity pathways.
- Ultimately, the data generated by these new tools are to be provided to risk assessors to use in the protection of human health and the environment.
- Key sections:
  - Toxicity Pathways
  - Chemical Selection
  - Analysis and Bioinformatics
  - Governance
  - Scientific Review
  - Outreach

Tox21 Goals

- Research, develop, validate, and translate innovative compound testing methods that characterize toxicity pathways
- Identify compounds, assays, informatic tools, and targeted testing needed for the innovative testing methods
- Prioritize compounds for more extensive toxicological evaluation
- Identify mechanisms of compound-induced biological activity in order to characterize toxicity pathways, facilitate cross-species extrapolation, and provide input to models for low-dose extrapolation
- Develop predictive models for biological response in humans



Tox21 Working Groups

- **Assays – K. Gaido (FDA), K. Houck (EPA), K. Witt (NTP), M. Xia (NCGC)**
  - Identify key toxicity pathways and assays for those pathways and prioritize assays for use at the NCGC
  - Identify methods for incorporating hepatic metabolism into *in vitro* assays
  - Consider approaches for evaluating compound, pathway, and cell-to-cell interactions
- **Compounds - D. Mendrick (FDA), A. Richard (EPA), N. Southall (NCGC), C. Smith (NTP)**
  - Establish a library ~10,000 compounds with known structures for screening at the NCGC
  - Establish procedures for determining the identity, purity, and stability of each compound
  - Establish a library of water soluble compounds and mixtures for testing at the NCGC

Tox21 Working Groups

- **Informatics - R. Huang (NCGC), J. Fostel (NTP), R. Judson (EPA), W. Tong (FDA)**
  - Identify compounds to be used as plate duplicates based on chemical space and biological activity
  - Evaluate patterns of response and relationship to adverse health outcomes in experimental animals and humans
  - Evaluate consistency of response within and across assays/endpoints
  - Make all data publicly accessible (CEBS, PubChem, ACToR)
- **Targeted Testing –D. Benz (FDA), K. Crofton (EPA), M. DeVito (NTP), D. Gerhold (NCGC),**
  - Develop strategies for evaluating the relevance of prioritization schemes and prediction models developed using biomolecular screening data
  - Prioritize substances for more complex testing, including the use of alternative assay platforms or species

NCGC Phase I NTP & EPA compound libraries

- NTP 1408 (1353 unique compounds, 55 duplicates)*
  - 1206 with NTP test data
  - 147 ICCVAM reference substances
  - MW = 32-1168, calculated log p = -3 to 13.2
- EPA: 1462 (1384 unique compounds, 78 duplicates)
  - MW = 58-516, calculated log p = -2.8 to 8.2
  - 400 compound overlap

qHTS assays used at the NCGC: Phase I

Apoptosis
- 3/7, 8, & 9

Cell Viability
- ATP
- LDH
- Protease release

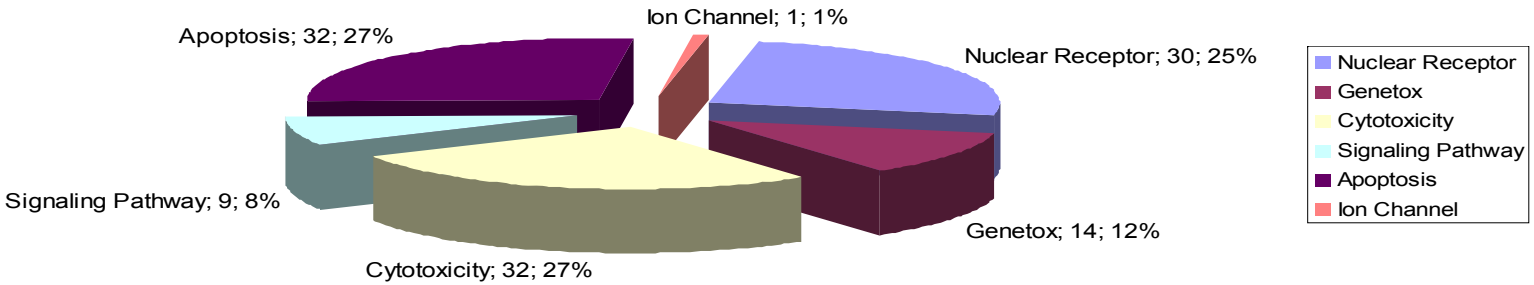
DNA damage
- ELG1
- p53
- Multiple repair-deficient DT40 cell lines

Epigenetics
- LDR

Mitochondrial toxicity

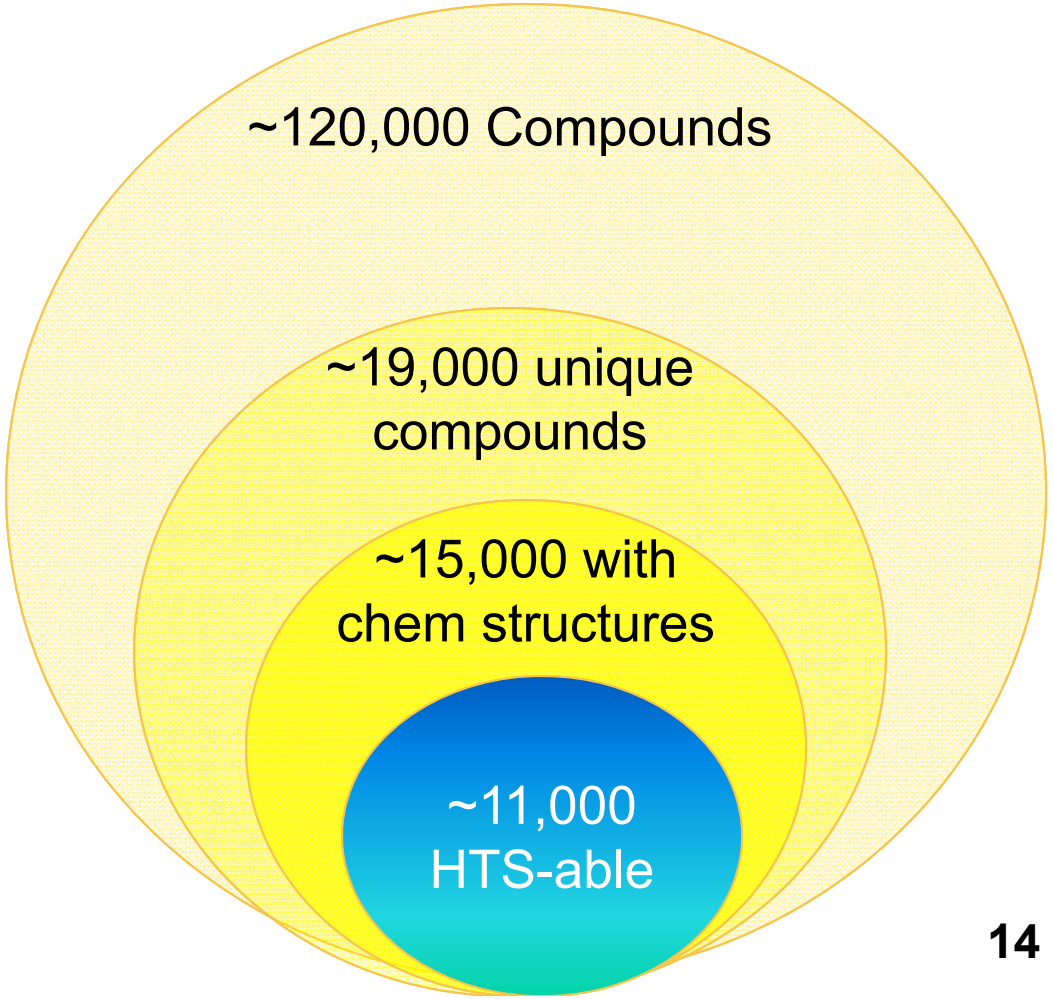
Nuclear Receptors
- hAR
- hERα
- hFXR
- hGR
- hLXRβ
- hPPARα
- hPPARγ
- hPPARδ
- hPXR
- rPXR
- hROR
- hRXR
- hTRβ
- hVDR

Pathways
- AP1
- ARE/Nrf2
- CRE
- hERG
- HRE
- HSP
- JNK3
- NFκB



Developing the "HTS-able" Library

- Removed duplicate/replicate chemicals
- Omitted compounds with no defined structures
- Used prediction models to screen for physical chemical properties (solubility, vapor pressure)
- Omitted undefined mixtures
- MW range about 100 – 1000
- Eventually about 11K compounds in "practical universe"



Tox21 10K Library

NCGC

- Drugs
- Drug-like compounds
- Active pharmaceutical ingredients

EPA

- ToxCast I and II compounds
- Antimicrobial Registration Program
- Endocrine Disruptor Screening Program
- OECD Molecular Screening Working Group List
- Failed Drugs

NTP

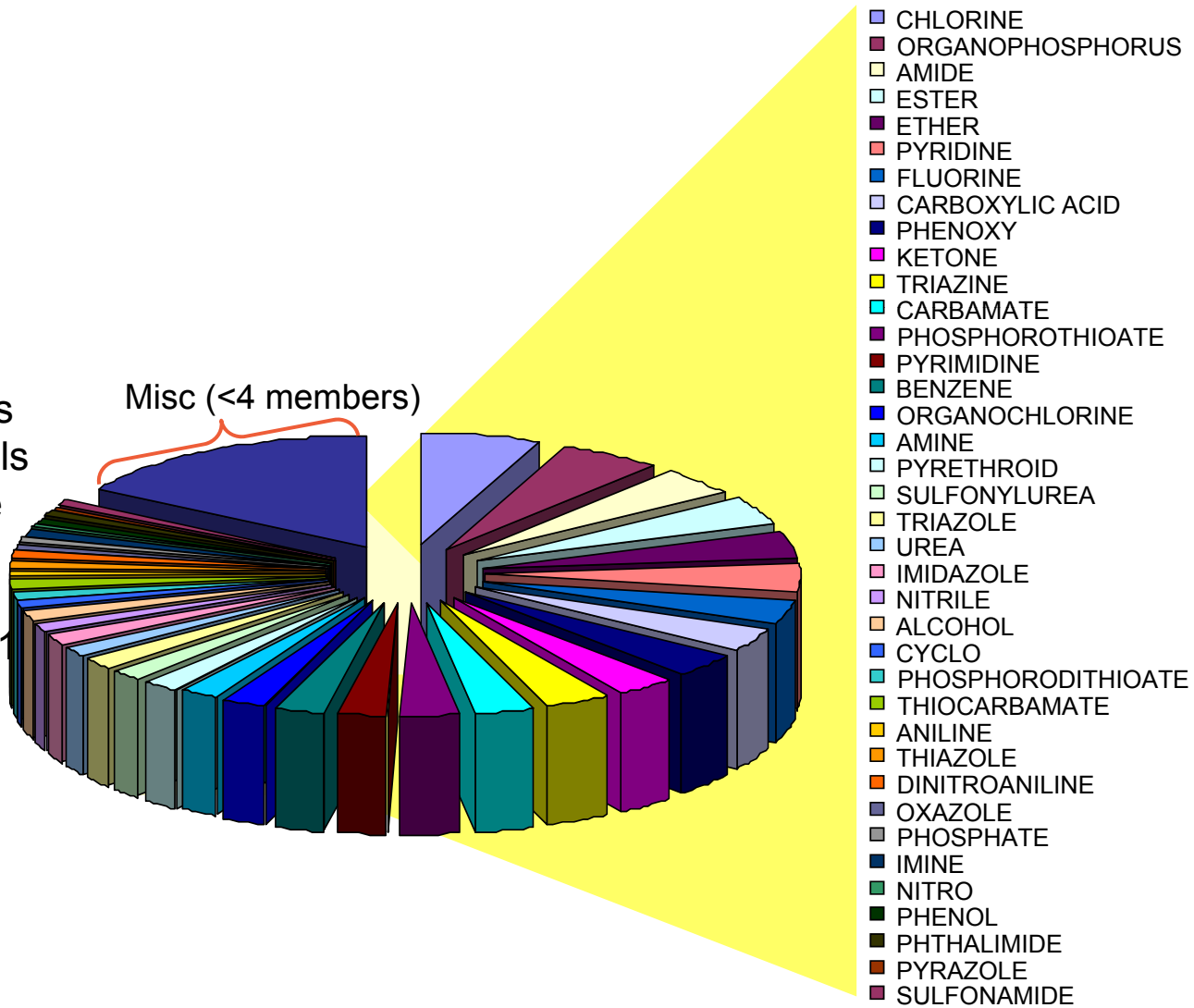
- NTP-studied compounds of all types
- NTP nominations and related compounds
- ICCVAM and NICEATM validation and reference compounds
- Outside collaborators (e.g., U.S. Army Public Health Command)

EPA's ToxCast™ Program

- Research program of EPA's National Center for Computational Toxicology (NCCT) (see http://www.epa.gov/ncct/toxcast)
- Addresses chemical screening and prioritization needs for EPA
- Comprehensive use of HTS technologies to generate biological fingerprints and predictive signatures
- Data released via ACToR (Aggregated Computational Toxicology Resource) (http://epa.gov/actor)
  - Contains data on ~500,000 environmental chemicals
  - Multiple Domains - Physchem, biological, use levels, regulations
  - Brings together data from >200 sources

The ToxCast™ Phase I Chemicals (320)

- 309 Unique Structures
- Replicates for QC
- 291 Pesticide Actives
- 9 Industrial Chemicals
- 13 Parent/Metablolite pairs
- 56/73 Proposed Tier 1 Endocrine Disruption Screening Program
- 14 High Production Volume Chemicals
- 11 HPV Challenge



ToxCast 1.0 (April, 2007)

- Enzyme inhibition/receptor binding HTS (Novascreen)
- NR/transcription factors (Attagene, NCGC)
- Cellular impedance (ACEA)
- Complex cell interactions (BioSeek)
- Hepatocelluar HCS (Cellumen)
- Hepatic, renal and airway cytotoxicity (IVAL)
- In vitro hepatogenomics (IVAL, Expression Analysis)
- Zebrafish developmental toxicity (Phylonix)

ToxCast 1.1 (January, 2008)

- Neurite outgrowth HCS (NHEERL)
- Cell proliferation (NHEERL)
- Zebrafish developmental toxicity (NHEERL)

ToxCast 1.2 (June, 2008)

- NR Activation and translocation (CellzDirect)
- HTS Genotoxicity (Gentronix)
- Organ toxicity; dosimetry (Hamner Institutes)
- Toxicity and signaling pathways (Invitrogen)
- *C. elegans* WormTox (NIEHS)
- Gene markers from microscale cultured hepatocytes (MIT)
- 3D Cellular microarray with metabolism (Solidus)
- Zebrafish vascular/cardiotoxicity (Zygogen)
- HTS stress response (NHEERL+NCGC)

Phased Development of ToxCast™

Phase	Number of Chemicals	Chemical Criteria	Purpose	Number of Assays	Target Date
I	*320*	*Data Rich (pesticides)*	*Signature Development*	*554*	*FY08*
Ib	15	Nanomaterials	Pilot	166	FY09
IIa	>300	Data Rich Chemicals	Validation	>400	FY10
IIb	**>100**	**Known Human Toxicants**	**Extrapolation**	**>400**	**FY10**
IIc	>300	Expanded Structure and Use Diversity	Extension	>400	FY10
IId	>12	Nanomaterials	PMN	>200	FY10
Iie	**50 NTP**	**Immunotoxicants**	**Signature development**	**>400**	**FY10**
III	Thousands	Data poor	Prediction and Prioritization	>300	FY 12

Other NTP Activities in Support of Tox21 (1)

- Support the development of assays and informatic tools through the NIEHS SBIR/STTR program
  - qHTS (e.g., gap junctions, ROS)
  - *in vitro* 3D tissue models (skin, lung, kidney)
  - lower organism models (*C. elegans*, zebrafish)
  - NexGen tools for archived tissues
  - informatic tools
- Linking basic research to Tox21 via collaborations with NIEHS intramural scientists
  - Dr. Perry Blackshear (TTP)
  - Dr. Anton Jetten (ROR)
  - Dr. Samuel Wilson (DNA repair deficient mouse cell lines)

Other NTP Activities in Support of Tox21 (2)

- Collaborating with scientists by providing the NTP 1408 compound library
  - Dr. Eileen Jaffe (Fox Chase Cancer Center, Philadelphia, PA) as part of a project on porphobilinogen synthase hexamer, disease states, and environmental contaminants
- Collaborating with scientists by providing samples of treated cells from the NCGC
  - Dr. Art Petronis & Viviane Labrie (the Krembil Family Epigenetics Laboratory Centre for Addiction and Mental Health, Toronto, Canada) to evaluate epigenetic alterations in cells exposed to hypo- and hyper-methylating compounds, based on an evaluation of the unmethylated fraction of DNA
- Collaborating with scientists to further characterize qHTS results
  - Dr. Craig Beeson (Medical University of South Carolina, Charleston, SC) for follow-up studies on mitochondria toxicants in the NTP 1408 compound library

Other NTP Activities in Support of Tox21 (3)

- **Human and Rodent Susceptibility studies**
  - Host Susceptibility Branch will provide lymphoblastoid cell lines, embryonic fibroblasts, and/or primary hepatocytes from >30 mouse strains
  - David Threadgill (NCSU) will provide iPS cells produced from embryonic fibroblasts obtained from Collaborative Cross RI mouse strains for use at the NCGC
  - In collaboration with Ivan Rusyn (UNC), conducted qHTS cytotoxicity and caspase 3/7 studies at the NCGC on ~80 Hapmap lymphoblastoid cell lines (Utah cohort) using 240 compounds selected on the basis of demonstrated cytotoxic in several cell lines.
  - Collaboration being expanded to 1000 lymphoblastoid cell lines screened for cytotoxicity against 180 compounds.

- **DrugMatrix Toxicogenomics Database**

A Tox21 Perspective (1)

- Teams of people who WANT to collaborate are essential.
- International scientific community participation is important.
- Process must be science-driven
  - chemicals ↔ genes ↔ pathways ↔ disease
- Technology matters; new technologies are frequently created to address needs.
- Making all data publicly available is critical.
- Large scale data is hypothesis generating.

A Tox21 Perspective (2)

- Prioritization and prediction depends on comprehensive suites of *in vitro* and *in vivo* assays in a tiered approach.
- Targeted animal tests (e.g., inbred strains of mice that have been densely sequenced) are needed to complement and demonstrate the relevance of *in vitro* tests.
- Computational models of toxicity pathways are necessary.
- Human toxicity data is essential if predictive models of human disease are to be demonstrated to be relevant.